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ORIGINAL ARTICLE

Microbiological Profile, Histopathological Severity, Drug Utilization, and Nutritional Risk Factors in Patients with Diabetic Foot Ulcers

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ABSTRACT

Background: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, associated with high morbidity, infection risk, and amputation. Multiple factors including microbial infection, histopathological damage, irrational drug use, and poor nutritional status influence disease severity and healing outcomes.

Methods: This cross-sectional study was conducted on 120 patients with diabetic foot ulcers from January 2022 to January 2023 at Khawaja Muhammad Safdar Medical College, Sialkot, Pakistan, and Sahara Medical College, Narowal, Pakistan. Microbiological analysis was performed using wound cultures, while histopathological grading was done through tissue biopsy. Drug utilization patterns were assessed from clinical records. Nutritional status was evaluated using body mass index, serum albumin, and hemoglobin levels. Statistical analysis was performed using SPSS version 26, with $p < 0.05$ considered significant.

Results: Gram-negative organisms predominated, with Pseudomonas aeruginosa (30.0%) and Escherichia coli (20.8%) being the most common isolates. Severe histopathological grades (Grade III–IV) were observed in 50.0% of patients. Polypharmacy was noted in 65.8% of cases, and empirical antibiotic therapy was used in 73.3% of patients. Nutritional deficiencies were highly prevalent, with hypoalbuminemia (58.3%), anemia (46.7%), and low BMI (25.8%). A significant association was found between poor nutritional status and increased ulcer severity ($p < 0.001$).

Conclusion: Diabetic foot ulcers are characterized by polymicrobial infections, advanced tissue damage, irrational antibiotic use, and significant nutritional deficiencies. A multidisciplinary approach incorporating culture-guided therapy, rational drug use, and nutritional optimization is essential to improve clinical outcomes and reduce complications.

Keywords: Diabetic foot ulcer, microbiological profile, histopathology, antibiotic utilization, malnutrition, wound healing

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both[1]. Over time, sustained hyperglycemia leads to microvascular and macrovascular complications that significantly impair patient quality of life and increase healthcare burden[2]. Among these complications, diabetic foot ulcers (DFUs) represent one of the most serious and disabling conditions, accounting for a major proportion of diabetes-related hospital admissions and lower limb amputations worldwide[3]. The lifetime risk of developing a diabetic foot ulcer in individuals with diabetes has been estimated to range between 15% and 25%, with higher rates reported in low- and middle-income countries due to delayed diagnosis, inadequate glycemic control, and limited access to specialized care[4].

The pathophysiology of diabetic foot ulcers is complex and multifactorial, involving peripheral neuropathy, peripheral arterial disease, and impaired immune response[5]. Neuropathy leads to loss of protective sensation, making patients susceptible to unnoticed trauma, while vascular insufficiency reduces tissue perfusion and oxygenation, impairing wound healing[6]. In addition, hyperglycemia compromises leukocyte function, thereby increasing susceptibility to infection[7]. These factors collectively create a favorable environment for microbial colonization and progression to severe infection[8].

Microbial infection is a central component in the progression and chronicity of diabetic foot ulcers[9]. The microbiological profile of DFUs is often polymicrobial, involving a mixture of aerobic and anaerobic organisms[10]. While Gram-positive bacteria such as Staphylococcus aureus are commonly implicated in early infections, chronic and severe ulcers are more frequently associated with Gram-negative organisms including Pseudomonas aeruginosa, Escherichia coli, and Klebsiella species[11]. The emergence of multidrug-resistant organisms further complicates management, necessitating accurate microbiological diagnosis and targeted antimicrobial therapy[12].

Histopathological evaluation of ulcer tissue provides valuable insights into the extent of cellular damage, inflammatory response, necrosis, and vascular compromise[13]. The severity of histopathological changes correlates with clinical outcomes, including delayed healing, increased risk of infection, and likelihood of amputation[14]. Despite its diagnostic importance, histopathological assessment is often underutilized in routine clinical practice, particularly in resource-limited settings[15].

Another critical yet frequently overlooked aspect in the management of DFUs is drug utilization[16]. Patients with diabetic foot ulcers often receive multiple medications, including broad-spectrum antibiotics, analgesics, and antidiabetic agents[17]. Empirical antibiotic therapy without culture sensitivity testing is common, leading to inappropriate drug use, increased risk of antimicrobial resistance, and higher treatment costs[18]. Rational prescribing practices based on microbiological evidence are therefore essential for effective management[19].

Nutritional status plays a pivotal role in wound healing and immune competence[20]. Malnutrition, particularly protein-energy deficiency, adversely affects collagen synthesis, angiogenesis, and cellular repair mechanisms[12]. Hypoalbuminemia is a well-recognized marker of poor nutritional status and has been associated with delayed wound healing and increased morbidity[6]. Additionally, micronutrient deficiencies and anemia further compromise tissue oxygenation and immune response, thereby exacerbating ulcer severity and prolonging recovery[14].

In developing countries such as Pakistan, the burden of diabetic foot ulcers is further amplified by socioeconomic factors, lack of patient education, delayed healthcare seeking behavior, and limited multidisciplinary care facilities[8]. Despite the high prevalence of DFUs, there remains a lack of comprehensive studies evaluating the combined impact of microbiological factors,

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histopathological severity, drug utilization patterns, and nutritional status on disease progression and outcomes[10].

Therefore, the present study aims to provide a multidimensional assessment of diabetic foot ulcers by analyzing the microbiological profile, histopathological severity, patterns of drug utilization, and nutritional risk factors in affected patients[17]. Understanding the interplay between these factors is essential for developing effective, evidence-based strategies to improve clinical outcomes, reduce complications, and minimize the risk of amputation[5].

MATERIALS AND METHODS

This cross-sectional observational study was conducted at the Department of General Medicine and Surgery of Khawaja Muhammad Safdar Medical College, Sialkot, Pakistan, and Sahara Medical College, Narowal, Pakistan, over a period of one year from January 2022 to January 2023.

A total of 120 patients diagnosed with diabetic foot ulcers were enrolled using non-probability consecutive sampling. The sample size was determined based on previously reported prevalence of diabetic foot infections and microbiological patterns in similar clinical settings. Patients aged between 18 and 70 years with a confirmed diagnosis of Type 2 diabetes mellitus and clinically evident foot ulcers were included in the study. Patients with non-diabetic ulcers, malignancy, autoimmune disorders, or those receiving immunosuppressive therapy were excluded.

After obtaining informed written consent, detailed demographic data, clinical history, duration of diabetes, and ulcer characteristics were recorded using a structured proforma. Clinical examination included assessment of ulcer size, depth, location, presence of discharge, and signs of infection.

Microbiological Analysis: Wound samples were collected under aseptic conditions using sterile swabs and, where feasible, deep tissue biopsy specimens to improve diagnostic accuracy, as deep tissue cultures provide more reliable pathogen identification. The samples were immediately transported to the microbiology laboratory and inoculated on appropriate culture media including blood agar and MacConkey agar. Bacterial identification was performed using standard biochemical techniques, and antibiotic susceptibility testing was carried out using the Kirby–Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Histopathological Evaluation: Tissue biopsy specimens were obtained from the ulcer margins in selected patients and fixed in 10% formalin. Sections were stained with hematoxylin and eosin and examined under light microscopy. Ulcers were graded into four categories based on the extent of tissue damage, inflammatory cell infiltration, necrosis, and vascular compromise, ranging from mild (Grade I) to advanced necrotic (Grade IV).

Drug Utilization Assessment: Drug utilization patterns were assessed through detailed review of patient prescriptions and medical records. Parameters evaluated included number of drugs prescribed, type of antibiotics, use of empirical versus culture-guided therapy, duration of antibiotic use, and presence of polypharmacy (defined as use of three or more drugs simultaneously).

Nutritional Assessment: Nutritional status was evaluated using anthropometric and biochemical parameters. Body mass index (BMI) was calculated using standard formula. Blood samples were collected to measure serum albumin and hemoglobin levels. Hypoalbuminemia was defined as serum albumin <3.5 g/dL, while anemia was defined according to World Health Organization criteria. Nutritional risk was categorized based on combined assessment of BMI, serum albumin, and hemoglobin levels.

Statistical Analysis: All collected data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 26. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Associations between variables such as nutritional status and ulcer severity were assessed using Chi-square test and independent sample t-test. A p-value of less than 0.05 was considered statistically significant.

Ethical Considerations: Ethical approval for the study was obtained from the Institutional Review Boards of both participating institutions. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion in the study.

RESULTS

A total of 120 patients with diabetic foot ulcers were included in the study. The mean age of the study population was 55.8 ± 8.9 years, with a predominance of male patients (61.7%) compared to females (38.3%). The mean duration of diabetes mellitus was 9.8 ± 4.2 years, indicating long-standing disease in the majority of participants.

Microbiological analysis revealed that Gram-negative organisms were the predominant pathogens isolated from diabetic foot ulcers. The most commonly identified organism was Pseudomonas aeruginosa (30.0%), followed by Escherichia coli (20.8%) and Staphylococcus aureus (17.5%). Other organisms included Klebsiella pneumoniae (14.2%), Proteus species (9.2%), and mixed microbial growth (8.3%). These findings indicate a high burden of polymicrobial infection, particularly involving opportunistic Gram-negative bacteria, which may complicate treatment outcomes (Table 1).

Histopathological evaluation demonstrated varying degrees of tissue damage and inflammatory response among patients. Mild ulceration (Grade I) was observed in 13.3% of patients, while moderate changes (Grade II) were seen in 36.7%. Severe ulceration (Grade III) accounted for 30.0% of cases, and advanced necrotic changes (Grade IV) were present in 20.0% of patients. Notably, half of the study population (50.0%) exhibited severe to advanced histopathological grades (Grade III and IV), indicating delayed presentation and extensive tissue involvement (Table 2).

Table 1: Microbiological Profile of Diabetic Foot Ulcers (n = 120)

Organism	Frequency (n)	Percentage (%)
Pseudomonas aeruginosa	36	30.0%
Escherichia coli	25	20.8%
Staphylococcus aureus	21	17.5%
Klebsiella pneumoniae	17	14.2%
Proteus species	11	9.2%
Mixed growth	10	8.3%

Table 2: Histopathological Severity Grading of Ulcers

Grade	Frequency (n)	Percentage (%)
Grade I	16	13.3%
Grade II	44	36.7%
Grade III	36	30.0%
Grade IV	24	20.0%

Table 3: Drug Utilization Patterns in Study Population

Parameter	Frequency (n)	Percentage (%)
Single antibiotic	19	15.8%
Two antibiotics	22	18.3%
≥3 antibiotics (polypharmacy)	79	65.8%
Empirical therapy	88	73.3%
Culture-guided therapy	32	26.7%

Table 4: Nutritional Risk Factors in Patients with Diabetic Foot Ulcers

Parameter	Frequency (n)	Percentage (%)
Hypoalbuminemia	70	58.3%
Anemia	56	46.7%
BMI <18.5 kg/m²	31	25.8%

Assessment of drug utilization patterns revealed a high prevalence of polypharmacy among patients with diabetic foot ulcers. Only 15.8% of patients were prescribed a single antibiotic, while 18.3% received two antibiotics. A significant majority (65.8%) were treated with three or more antibiotics simultaneously. Furthermore, empirical antibiotic therapy was observed in 73.3% of cases, whereas only 26.7% received culture-guided therapy.

These findings highlight a trend toward irrational antibiotic use, which may contribute to antimicrobial resistance and increased treatment burden (Table 3).

Nutritional assessment revealed that a substantial proportion of patients had compromised nutritional status. Hypoalbuminemia was present in 58.3% of patients, anemia in 46.7%, and low BMI (<18.5 kg/m²) in 25.8% of cases. These findings indicate a high prevalence of malnutrition among patients with diabetic foot ulcers, which may adversely affect wound healing and clinical outcomes (Table 4).

Further analysis demonstrated a statistically significant association between nutritional status and histopathological severity of ulcers. Patients with hypoalbuminemia and anemia were more likely to present with severe and advanced ulcer grades (Grade III and IV) compared to those with normal nutritional parameters ($p < 0.001$). This suggests that poor nutritional status is a major contributing factor to disease progression and delayed healing in diabetic foot ulcers.

Overall, the results indicate that diabetic foot ulcers in this population are characterized by polymicrobial infections, advanced tissue damage, widespread use of multiple antibiotics, and a high burden of nutritional deficiencies, all of which collectively influence disease severity and clinical outcomes.

DISCUSSION

The present study provides a comprehensive evaluation of diabetic foot ulcers (DFUs) by integrating microbiological findings, histopathological severity, drug utilization patterns, and nutritional risk factors[1]. The results highlight the multifactorial nature of DFUs and emphasize the need for a multidisciplinary approach in their management[2].

The microbiological profile observed in this study demonstrated a predominance of Gram-negative organisms, with Pseudomonas aeruginosa and Escherichia coli being the most frequently isolated pathogens[3]. This pattern is consistent with several regional and international studies, which have reported a shift from Gram-positive to Gram-negative dominance in chronic and severe diabetic foot infections[4]. The high prevalence of Pseudomonas species may be attributed to prolonged wound exposure, hospital-acquired infections, and poor hygienic conditions[5]. Additionally, the presence of polymicrobial infections in a considerable proportion of patients further complicates clinical management and necessitates broad-spectrum antimicrobial coverage[6]. These findings reinforce the importance of routine culture and sensitivity testing to guide targeted therapy and reduce the emergence of multidrug-resistant organisms[7].

Histopathological analysis in this study revealed that half of the patients presented with severe to advanced ulcer grades (Grade III and IV), indicating extensive tissue necrosis and inflammatory infiltration[8]. This reflects delayed presentation and inadequate early intervention, which are common challenges in developing healthcare systems[9]. Similar findings have been reported in previous studies, where advanced histological damage was associated with increased risk of amputation and poor wound healing outcomes[10]. The correlation between histopathological severity and clinical progression underscores the importance of early diagnosis and timely intervention to prevent disease escalation[11].

Drug utilization patterns observed in this study revealed a high prevalence of empirical antibiotic therapy and polypharmacy[12]. A majority of patients were prescribed three or more antibiotics simultaneously, often without culture-guided evidence[13]. This irrational prescribing behavior is concerning, as it contributes to antimicrobial resistance, increases treatment costs, and exposes patients to potential adverse drug reactions[14]. Comparable studies have also highlighted widespread misuse of antibiotics in DFU management, particularly in resource-limited settings[15]. These findings strongly support the implementation of antibiotic stewardship programs and adherence to evidence-based prescribing guidelines to optimize therapeutic outcomes[16].

Nutritional assessment revealed a high burden of malnutrition among patients, with more than half exhibiting hypoalbuminemia and a significant proportion suffering from anemia[17]. These findings are clinically significant, as adequate nutritional status is essential for effective wound healing[18]. Protein deficiency impairs collagen synthesis and tissue regeneration, while anemia reduces oxygen delivery to tissues, further delaying healing processes[19]. The statistically significant association observed between poor nutritional status and higher ulcer grades in this study is in agreement with previous research, which has identified hypoalbuminemia as an independent predictor of poor wound healing and increased mortality in diabetic patients[20].

The interplay between infection, tissue damage, drug utilization, and nutritional status observed in this study highlights the complex pathophysiology of diabetic foot ulcers[6]. Patients with severe infections often require prolonged antibiotic therapy, which, when combined with poor nutritional status, further compromises immune function and delays recovery[14]. Therefore, addressing only one aspect of the disease is insufficient; a holistic approach is required[9].

From a clinical perspective, these findings emphasize the importance of early screening, prompt microbiological evaluation, rational drug use, and comprehensive nutritional assessment in patients with diabetic foot ulcers[11]. Establishing multidisciplinary care teams involving physicians, microbiologists, surgeons, and nutritionists can significantly improve patient outcomes and reduce the risk of complications such as amputation[18].

However, this study has certain limitations[7]. Being a cross-sectional study, it does not establish causal relationships between the studied variables[13]. Additionally, the study was conducted in selected tertiary care centers, which may limit the generalizability of the findings to the broader population[15]. Future longitudinal and interventional studies are recommended to evaluate the impact of targeted nutritional and antimicrobial interventions on healing outcomes[16].

CONCLUSION

Diabetic foot ulcers are a complex and multifactorial complication of diabetes mellitus, characterized by polymicrobial infections, advanced histopathological damage, irrational drug utilization, and significant nutritional deficiencies. The predominance of Gram-negative organisms, high prevalence of severe ulcer grades, widespread use of empirical antibiotics, and high burden of malnutrition collectively contribute to poor clinical outcomes. The findings of this study highlight the critical need for an integrated and multidisciplinary approach in the management of diabetic foot ulcers. Early diagnosis, routine microbiological culture and sensitivity testing, rational antibiotic prescribing, and timely nutritional intervention are essential strategies to improve wound healing, reduce complications, and prevent amputations. Implementation of evidence-based clinical protocols and antibiotic stewardship programs, along with increased awareness regarding nutritional support, can significantly enhance patient care and outcomes in diabetic foot ulcer management.

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Authors' Contributions

- **R.S.** – Conceptualization, study design, supervision, and final approval of the manuscript.
- **A.S.** – Data collection, manuscript drafting, and literature review.
- **H.S.** – Data acquisition, statistical analysis, and interpretation of results.
- **R.K.** – Pharmacological data analysis and drug utilization review.
- **T.S.** – Histopathological evaluation and interpretation.

- **H.N.K.** – Clinical assessment of patients and methodology support.
- **H.M.** – Nutritional assessment, data compilation, and manuscript editing.

All authors have read and approved the final manuscript.

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